ISOLATION AND PROPERTIES OF GROUP SPECIFIC 2-OXOGLUTARATE AMINOTRANSFERASE FROM *STREPTOMYCES NOURSEI* 2/9

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1. Introduction

Enzyme activities that catalyze transamination between 2-oxoglutarate and amino acids are widely distributed. Thus, Feldman and Gunsalus [1] demonstrated the formation of glutamate from 2-oxoglutarate and wide range of amino acids with cell suspension of *Escherichia coli* and *Pseudomonas fluorescens*. Similar results were obtained with preparations of *Neurospora crassa* [2] and *Salmonella typhosa* [3].

Evidence has been reported for the existence of separate 2-oxoglutarate aminotransferases. Rudman and Meister [4] have described the isolation and properties of two 2-oxoglutarate transaminases from *E. coli*, possessing higher degrees of specificity. Transaminase B catalyzed a reaction between 2-oxoglutarate and valine, leucine, isoleucine, methionine, phenylalanine and lysine. The present paper reports the purification and properties of a group-specific 2-oxoglutarate aminotransferase from *Streptomyces noursei*.

2. Materials and methods

The strain *S. noursei* 2/9 was cultivated on synthetic media as described by Půža et al. [5] and harvested after 72 hr. The yield of cells was approximately 60 g/l. Cells washed three times with 0.02 M K-phosphate buffer pH 7.8 were homogenized by X-press (Biox Nacka, Sweden) at −60°C. The homogenate was resuspended in 0.02 M K-phosphate buffer pH 7.8 and gently stirred for 30 min to thaw the homogenate. The enzyme preparation was then centrifuged for 30 min at 10,000 × g and the pellet discarded. The supernatant solution was used for further fractionation.

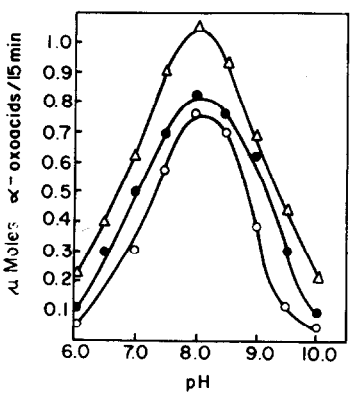

Fig. 1. Optimum pH of transamination 2-oxoglutarate valine – ○; leucine – ●; isoleucine – △.

Routine transamination assays were performed essentially as described by Wada and Snell [6]. The reaction was stopped by trichloroacetic acid (final concentration 5%) and the mixture centrifuged at 4,000 × *g* for 10 min. The supernatant was mixed with 0.5 ml of a 1% solution of 2,4-dinitrophenylhydrazine in 2 N HCl and placed in a 37°C water bath for 5 min. The 2,4-dinitrophenylhydrazone of the 2-oxoacid was extracted with 5 ml of 0.1 N HCl and then centrifuged at 4000 × *g* for 10 min. The aqueous phase was removed and the toluene phase mixed with 2 ml of 10% solution of sodium carbonate. One ml of the resulting solution was diluted with 1 ml of 4 N NaOH and the colour measured in a Unicam SP 700 Spectrophotometer at 450 nm.

Proteins were estimated by biuret reaction according to Weichselbaum [7]. The specific activity of the enzyme at any stage during the purification is expressed in μmoles of 2-oxoacid formed per mg of protein in 15 min.

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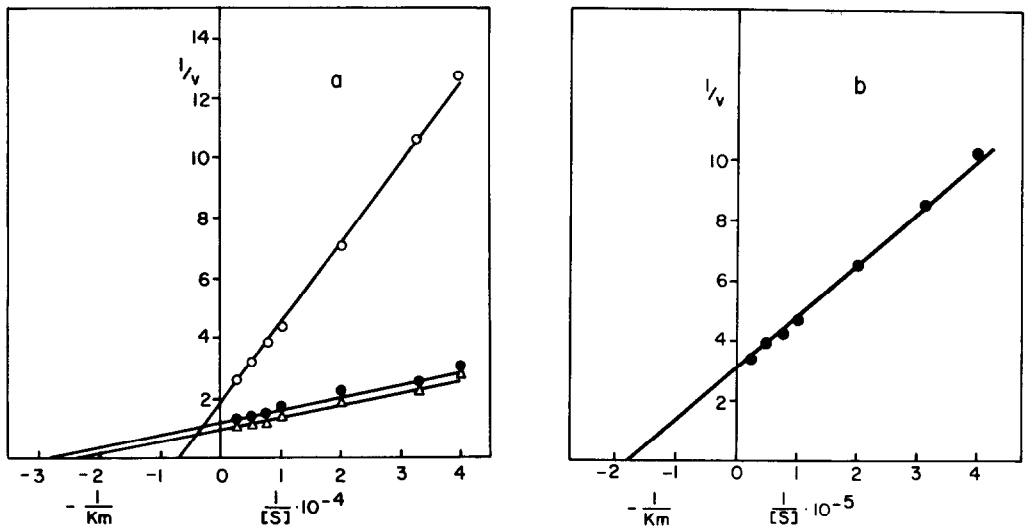

Fig. 2. The K_m values of 2-oxoglutarate aminotransferase a) for valine – ○; leucine – ●; isoleucine – △; b) for 2-oxoglutarate.

3. Results and discussion

The supernatant solution (fraction 1) was mixed with 30 ml of a 2% solution of protamine sulphate in 0.1 M K-phosphate buffer pH 7.8. After centrifugation at 15000 × *g* for 30 min the supernatant (fraction 2) was fractionated with ammonium sulphate. The precipitate between 40–70% saturation was solubilized in 0.01 M K-phosphate buffer pH 7.8 containing 1 μg pyridoxal phosphate/ml and dialyzed against the same buffer (fraction 3). The dialyzed enzyme was fractionated on the column of DEAE cellulose (2 × 30 cm) which was equilibrated with 0.01 M K-phosphate buffer containing 1 μg pyridoxal phosphate/ml. The enzyme was eluted with the same buffer containing 0.1 M KCl (fraction 4). During the elution a partial inactivation of the enzyme was encountered. The active fraction was refractionated with ammonium sulphate, the precipitate obtained at 50% saturation was centrifuged at 20000 × *g* for 30 min. The supernatant was discarded and the precipitate solubilized in 10 ml of 0.01 M K-phosphate buffer pH 7.8. The enzyme was then dialyzed against the same buffer and centrifuged to give the purified enzyme solution (fraction 5). All preparation processes were performed at 2°C. A summary of the purification procedure is given in table 1. Enzyme activity was estimated in a standard incubation mixture containing in μmoles: K-phosphate buffer pH 7.8 (200), L-aminoacids (20), 2-oxoglutarate (20), pyridoxal phosphate (10 μg), enzyme (100 μl), total volume 1.5 ml, temperature 37°C.

The purified enzyme was stable for 30 min at 40°C in 0.1 M K-phosphate buffer pH 7.8 with 1 μg/ml of pyridoxal phosphate. After heating the enzyme to 80°C for 5 min in presence of pyridoxal phosphate or substrates no activity was observed. The optimal pH range for all substrates lies between 7.8–8.2 (fig. 1). In measurements of activity for the purpose of K_m determination standard assay concentrations were used with the exception of the compound being tested. The values of K_m for valine (1.7×10^{-2}M), leucine (3.4×10^{-3}M) and isoleucine (3.9×10^{-3} M) (fig. 2), as well as for 2-oxoglutarate (0.58×10^{-4} M) (fig. 2b) were obtained by using the reciprocal plot of Dixon [8]. The enzyme is specific for valine, leucine and isoleucine. No transaminase activity was found with aspartic acid, methionine, alanine or tryptophane. This enzyme, therefore, differs in group specificity from the transaminase B of Rudman and Meister [4] which catalyzed transamination between 2-oxoglutarate and valine, leucine, isoleucine, methionine, alanine and phenylalanine.

Table 1
Purification of 2-oxoglutarate aminotransferase from *S. noursei.*

Fraction	Volume (ml)	Protein (mg/ml)	Spec. activity for isoleucine	Recovery (%)
1	150	31.2	0.029	100
2	170	10.2	0.050	68
3	35	14.1	0.1	36
4	20	6.1	0.89	8
5	10	4.9	2.02	7.3

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